IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor: Avner Yayon

Application No.: 10/734,661

Filing Date: December 15, 2003

For: ANTIBODIES THAT BLOCK RECEPTOR PROTEIN TYROSINE KINASE ACTIVATION, METHODS OF SCREENING FOR AND USES THEREOF

Confirmation No.: 5324

Patent No.: 7,498,416 B2

Patent Date: March 3, 2009

Attorney Docket No.: 81408-4400

REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 C.F.R. § 1.322

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

It is requested that a Certificate of Correction be issued in connection with the above-identified patent correcting the errors listed on the accompanying Form PTO-1050. The corrections requested are as follows:

At column 34, line 24, after "overnight at", delete "40°C" and insert -- 4°C --. Support for this change appears in the amended paragraph starting at page 41, line 24 in the substitute specification filed June 7, 2004.

At column 38, line 41, after "1 hour at", delete "40°C" and insert -- 4°C --. Support for this change appears in the amended paragraph starting at page 48, line 1 in the substitute specification filed June 7, 2004.

This request is being made pursuant to 37 C.F.R. § 1.322 since the errors are clerical or typographical in nature and appear to have been made by the Office during the printing of the patent. Therefore, no fee is believed to be due for this request. Should any fees be required, however, please charge such fees to Winston & Strawn LLP Deposit Account No. 50-1814.

Respectfully submitted,

4/22/09

Date

Allan A. Fanucci, Reg. No. 30,256

WINSTON & STRAWN LLP
Customer No. 28765

212-294-3311

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 7,498,416 B2
APPLICATION NO. : 10/734,661
DATED: : March 3, 1009
INVENTOR(S) : Yayon et al.

It is certified that an error appears or errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 34
Line 24, after "overnight at", delete "40°C" and insert -- 4°C --.

Column 38
Line 41, after "1 hour at", delete "40°C" and insert -- 4°C --.

WINSTON & STRAWN LLP
Patent Department
1700 K Street, N.W.
Washington, D.C. 20006-3817

Example 3

Screening for Antibodies

Panning and First Screening of Ab Binding Characterization

The screening strategies to identify Fabs from the Human Combinatorial Antibody Library (HUCAL®, developed at MorphoSys, Munich, Germany and disclosed in WO 97/08320, U.S. Pat. No. 6,300,064, and Knappik et al., (2000), the entire contents of which are incorporated herein by reference, using soluble dimeric forms of the extracellular domain of the FGFR3 receptor are shown in Table 2.

TABLE 2

Panning Strategies

	Panning Round 1	Panning Round 2	Panning Round 3
Screen 1	FR3-TDhis	HEK293	FR3-TDhis
Screen 2	FR3exFc captured with mouse anti-human IgG	RCJ-FR3ach	FR3exFc captured with mouse anti-human IgG
Screen 3	FR3-TDhis (Round 1 of panning 1)	RCJ-FR3ach & RCJ-FR3wt	FR3exFc Captured with mouse anti-human IgG

The screening was carried out, for example in Screen 1, by coating the wells of a 96 well plate with $hFR3^{23-374}$TDhis (FR3-TDhis), panning with the bacteriophage library and selecting the positive clones. The positive clones were then tested on HEK293 (293, human embryonic kidney) cells, expressing endogenous FGFR3. The positive clones were selected and rescreened on FR3-TDhis. Two additional similar screenings were carried out as shown in Table 2. In screen 2 the first and third pannings were carried out with the FR3exFc antigen and the second panning carried out with RCJ cells expressing a mutant (achondroplasia) form of FGFR3. An overview of the number of initial hits and of the candidate clones is shown in Table 3.

TABLE 3

Overview of Screenings 1, 2 and 3 on FGFR3

	screened clones	primary hits	sequenced clones	consolidated candidate clones (ELISA & FACS)
Screen 1	1076	208	69	15 (MSPRO 1-15)
Screen 2	864	300	32	22 (MSPRO 20-33 and 52-59)
Screen 3	768	487	52	11 (MSPRO 40-50)

Sequence and Vector Data

A plasmid map of the dHLX-MH vector having SEQ ID NO:52 is presented in FIG. **28**. FIG. **29** shows the plasmid map of the phage display vector, having SEQ ID NO:53, used in accordance with the present invention.

FIG. **30** displays the polynucleotide sequences of the specific V_L and V_H domains of MSPRO2 (SEQ ID NO:67 and 77); MSPRO11 (SEQ ID NO:63 and 78); MSPRO12 (SEQ ID NO:68 and 82); MSPRO21 (SEQ ID NO:60 and 71); MSPRO24 (SEQ ID NO:57 AND 72); MSPRO26 (SEQ ID NO:64 AND 79); MSPRO28 (SEQ ID NO:55 AND 73); MSPRO29 (SEQ ID NO:58 AND 80); MSPRO54 (SEQ ID NO:66 AND 75); MSPRO55 (SEQ ID NO:62 AND 76); and MSPRO59 (SEQ ID NO:69 AND 84). The sequences include the framework domains 1-4 and the CDR domains 1-3. SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:59, SEQ ID NO:61, and SEQ ID NO:65 denote herein the polynucleotide sequences of the parent V_L (kappa or lambda) strands. SEQ ID NO:70, SEQ ID NO:74, SEQ ID NO:81 and SEQ ID NO:83 denote herein the polynucleotide sequences of the V_H parent strands.

Example 4

Analysis of Fabs Identified in First Screening

Specificity of Antibody Recognition

The first screening yielded 15 different Fabs that specifically recognize FGFR3 in vitro and on the cell surface. Fourteen of these were further analysed. LY6.3, an anti-lysosyme antibody, was isolated from the same library and serves as a control. ELISA analysis, according to the following protocol was carried out to determine the specificity of the isolated Fabs for FGFR3 or FGFR1.

Fab-FR3/Fc Binding Assay

MAXISORP® ELISA plates were coated with 100 µl anti-human Fc (10 µg/ml) in bicarbonate overnight at 40° C. Wells were washed five consecutive times with a PBS solution containing 0.1% Tween 20 (PBST). The well surface was blocked with 250 µl PBST+3% BSA (blocking solution) for 1 hour at 37° C. This was followed by capturing 1 µg of FGFR/Fc for 1 hour at room temperature. To assess the antibody binding to the captured FGFR/Fc, 1 µg each of the tested Fabs was incubated in 100 µl blocking solution per well 1 hour at room temperature. Wells were washed 5 times with PBST. Reaction was initiated with the addition of 100 µl of 0.8 µg/ml goat anti-human Fab-HRP (horseradish peroxidase) diluted in blocking solution, subsequently washed and detected with TMB substrate (Pierce). The absorbance was measured at 450 nm. A comparison of ELISA analyses done in both laboratories, Prochon and MorphoSys, is presented in FIG. **27** and in Table 4.

TABLE 4

	ProChon		MorphoSys	
	FR1/Fc	FR3/Fc	FR1/Fc	FR3/Fc
MS-PRO1	++	++	+/−	+
MS-PRO2	−	++	−	++
MS-PRO3	+	++	−	++
MS-PRO4	−	+	−	++
MS-PRO5	−	++	+/−	+
MS-PRO6	−	++	−	+
MS-PRO7	−	++	−	+
MS-PRO8	+	++	−	+
MS-PRO9	−	+/−	+/−	+
MS-PRO10	+	++	−	++
MS-PRO11	−	+/−	+	++
MS-PRO12	−	+/−	−	++
MS-PRO13	−	+/−	+/−	+
MS-PRO14	−	−	−	+
LY6.3 (control)	−	−		

In most cases, the data generated in both laboratories are in agreement. However, some Fabs behave differently. For example, MS-PRO3 and MS-PRO-10 were found to be completely FGFR3-specific under certain conditions while under other conditions both show considerable cross-reaction with FGFR1. Subsequent FACS analysis supported the cross reactivity for MS-PRO3, but not for MS-PRO10. Taking into account the potency and specificity of the Fabs, MS-PRO2 had the highest score according to these preliminary data.

were added to FDCP-FR3 (closed triangle ? (2), star * (3), and circle ? (4)) or FDCP-FR1 (open triangle ? (2), open square ? (3) and open circle ? (4)) grown in the presence of FGF9. Two days later, an XTT proliferation assay was performed. While none of the Fabs inhibited FDCP-FR1 cell proliferation, MSPRO2 (?) and MSPRO3 (*) inhibited FDCP-FR3 proliferation with a similar IC50 of about 1.0 μg/ml. In contrast, MSPRO4 (?) had no inhibitory effect on FDCP-FR3 proliferation. The rest of the Fabs, MSPRO 1, 3, 5, 6, 7, 9, 11, 12, 13, 14, were similarly analyzed on FDCP-FR3 expressing cells. Increasing amounts of the indicated Fabs were added to FDCP-FR3 grown in the presence of FGF9 (FIG. **6**). Inhibitors of FGFR3 signaling were antibodies MSPRO 1, 3, 5, 7, 9, 11, 12. The results of the proliferation assay done at two sites are compared in Table 7 (NA=data not available).

TABLE 7

	Prochon		MorphoSys	
	FDCP-FR1	FDCP-FR3	FDCP-FR1	FDCP-FR3
MSPRO1	–	++	NA	NA
MSPRO2	–	++	NA	++
MSPRO3	–	++	NA	++
MSPRO4	–	–	NA	–
MSPRO5	–	+	NA	+
MSPRO6	–	–	NA	+/–
MSPRO7	–	++	NA	+
MSPRO8	–	+/–	NA	+/–
MSPRO9	–	+	NA	+
MSPRO10	–	+	NA	NA
MSPRO11	–	+++	NA	++
MSPRO12	–	+++	NA	+++
MSPRO13	–	–	NA	NA
MSPRO14	–	–	NA	NA
LY6.3	–	–	NA	NA

As shown in Table 7, there is an excellent agreement between the data. About half of the Fabs show considerable neutralizing activity, MSPRO12 being the most potent. Most of the inhibitory Fabs performed well in the binding assay (Table 4), with MSPRO11 and MSPRO12 being the exception to the rule, however, clearly remain good candidates to pursue. None of the Fabs (including those that crossreact with FGFR1) inhibited FGF-dependent FDCP-FR1 proliferation. In addition, FDCP-FR3 cells grown in the presence of IL-3 were not affected by any of the Fabs.

An additional 20 new Fabs were selected from the second panning. Three of these new Fabs were subjected to the FDCP cell proliferation test and all were found to neutralize the receptor (MSPRO52 (?), MSPRO54 (?) and MSPRO55 (*) in FIG. **7**A). Interestingly and in accord with MorphoSys affinity data, one Fab (MSPRO54) showed strong neutralizing activity against FGFR1 (FIG. **7**B). MSPRO29 (?) and a control antibody Ly6.3 (¦) were also tested in this assay.

Example 7

Receptor Expression and Activation in RCJ Cells

RCJ Cell Assay

RCJ cells (fetal rat calvaria-derived mesenchymal cells, RCJ 3.1C5.18; Grigoriadis, 1988) were generated to express various FGF Receptors in an inducible manner, in the absence of tetracycline. The M14 line (RCJ-FR3ach) expresses FGFR3-ach380 mutant upon induction by the removal of tetracycline. The cells were incubated in low serum after which FGF was added to stimulate receptor function and signaling. The cells were lysed and the receptor level, receptor activation and signaling are assessed by Western with anti-active ERK (or JNK) (Promega). The lysates is immunoprecipitated with anti-FGFR3 (Santa Cruz), and a Western immunoblots is prerformed using anti-phospho-tyrosine (Promega) antibodies. W11 refers to the RCJ cells expressing wild type FGFR3. RCJ-FR1 and RCJ-FR2 refer to RCJ cells expressing the FGFR1 and FGFR2 receptors, respectively. FIG. **21** provides a flow chart of the experimental procedure.

The transfected RCJ cells were grown in a-MEM supplemented with 15% fetal calf serum, 1× penicillin/streptomycin/nystatin, 1× glutamine, 600 μg/ml neomycin, 2 μg/ml tetracycline, 50 μg/ml hygromycin B to subconfluence. The medium was aspirated off and the cells washed with trypsin, 1 ml/10 cm dish, then trypsinized with 0.5 ml/10 cm dish. The cells were resuspended in 10 ml a-MEM supplemented with 15% fetal calf serum, 1× penicillin/streptomycin/nystatin, 1× glutamine, 600 μg/ml neomycin, and 2 μg/ml tetracycline.

Six hundred thousand (6×10^6) cells/well were seeded in a 6-well dish. The cells were washed thrice 24 hours later (or 8 hours later if twice the amount of cells are seeded) with 1 ml a-MEM, and then incubated with a-MEM supplemented with 15% fetal calf serum, 1× penicillin/streptomycin/nystatin, and 1× glutamine (induction medium) for 16 hours. Cells were washed thrice with 1 ml a-MEM and allowed to grow for 4 additional hours in 1 ml of 0.5% exhausted serum (prepared by diluting the induction medium X30 with a-MEM).

FGF9 (1 ng/ml) was added for 5 minutes and the cells placed on ice. The cells were washed twice with ice-cold PBS and lysed with 0.5 ml lysis buffer. The cells were scraped into an Eppendorf tube, vortexed once and placed on ice for 10 minutes. The lysate was microcentrifuged for 10 minutes at 4° C., and the cleared lysate was transferred into a fresh Eppendorf tube.

The protein content was determined by Bradford or DC protein assay (Bio-Rad, cat# 500-0116) following manufacture instructions. Total protein aliquots, supplemented with 1/5 volume of 5× sample buffer, were boiled for 5 minutes and stored at −20° C. until ready to load on gel. In parallel an immunoprecipitation (IP) assay was performed, 10 μl anti-FGFR3 antibodies were added to the rest of the lysates and incubated for 4 hours at 40° C. Twenty (20) ul protein A-SEPHAROSE® was added and incubated for 1 hour at 4° C. with continuous shaking. Afterwards, the mixture was microcentrifuged 15 seconds, and the fluid was aspirated, carefully leaving a volume of ~30 μl above the beads. The beads were washed 3 times with 1 ml lysis buffer. At this step, the protease inhibitor mix was omitted from the buffer. 4°C

After the final wash, 15 μl of 5× sample buffer was added, samples were boiled 5 minutes and stored at −20° C. until ready to load onto gel. Samples were loaded onto a 7.5% SDS-PAGE, cast on a Mini-PROTEAN II electrophoresis cell, and run at 100 V through the upper gel and at 150 V through the lower gel. Proteins were transferred onto a nitrocellulose sheet using the Mini trans-blot electrophoretic transfer cell at 100 V for 75 minutes or at 15 V overnight. The lower part of the total lysate Western blots was probed with anti-active JNK (anti-phosphorylated Jun Kinase) and the upper part was probed with anti-FGFR3, both at 5×10^3 dilutions.

FIG. **8**A shows that MSPRO2 blocks FGFR3 activation in W11 cells and weakly blocks signaling in M14 cells, and MSPRO12 blocks FGFR3 receptor activation in W11 and M14 expressing cells. Furthermore MSPRO13 appeared to be able to block FGFR1 activation while none of the Fabs blocked FGFR2 activation. FIG. **8**B shows the inhibitory capacity of MSPRO12 and MSPRO59 on wild type FGFR3 expressing cells, as seen as reduction in JNK signaling.